Homoazanicotine: A Structure-Affinity Study for Nicotinic Acetylcholine (nACh) Receptor Binding

G. Ferretti,[†,‡] M. Dukat,[‡] M. Giannella,[†] A. Piergentili,[†] M. Pigini,[†] W. Quaglia,[†] M. I. Damaj,[§] B. R. Martin,[§] and R. A. Glennon*[,‡,§]

Dipartimento di Scienze Chimiche, Universita di Camerino, 6032 Camerino, Italy, Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, Virginia 23298, and Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia 23298

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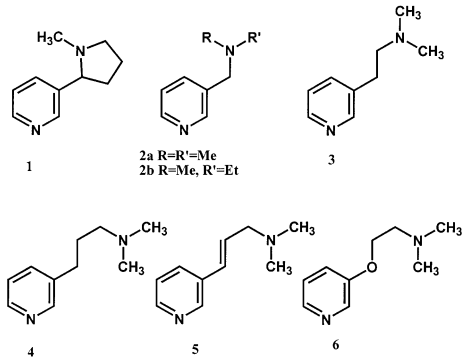
We have recently identified 3-[(1-methyl)-4,5-dihydro-1H-imidazol-2-yl)methyl]pyridine (homoazanicotine, **8**) as a novel nicotinic acetylcholinergic (nACh) receptor ligand. In the present investigation, after we determined that **8** binds selectively at nicotinic (K_i = 7.8 nM) vs muscarinic (K_i > 10 000 nM) acetylcholinergic receptors, we examined its structure−affinity relationships for nACh receptor binding. The features investigated included the influence of (i) the composition of connector that separates the two rings, (ii) the *N*-methyl group, (iii) the ring opening of the imidazoline ring, (iv) the pyridine nitrogen atom, and (v) the aromatization of the imidazoline ring on nACh receptor affinity. As with nicotine, the parent structure seems optimal and most structural changes reduce nACh receptor affinity. Also, as with nicotine analogues, alteration of the spacer group influences affinity in a manner that is somewhat different than that seen with the parent structure.

Introduction

Cholinergic receptors are one of the oldest known populations of neurotransmitter receptors, and acetylcholine is believed to produce most of its actions by acting either at muscarinic (mACh) or nicotinic acetylcholinergic (nACh) receptors. The past twenty years have witnessed a remarkable resurgence of interest in nACh receptors,[1] including a considerable shift from an investigation of peripheral nACh receptors to central or neuronal ACh receptors, for several reasons. It was during this time that techniques and radioligands were developed for studying brain nicotinic receptors.[1] There also exists today a better understanding of the structure of cholinergic receptors,[2,3] that neuronal nACh receptors can be structurally different from peripheral nACh receptors,[2] and that their structural composition likely dictates their pharmacology and binding characteristics.[1,3] The major population of nACh receptors in mammalian central nervous system (CNS) appears to be of the $\alpha 4\beta 2$ type, and evidence suggests that neuronal nicotinic receptors are involved in appetite, memory, analgesia, and various other physiological processes as well as in anxiety and certain mental and neurological disorders.[4] Although nicotine (**1**), a naturally occurring nACh receptor ligand, is associated with a variety of toxic side effects,[4] there is no reason to believe that these side effects are inextricably linked to the beneficial effects of nicotinic ligands. This has prompted an investigation of structure−affinity relationships (SAFIR) for central nACh receptor binding, and structure−activity relationships (SAR) for nicotinic agonist and antagonist activity, to formulate pharmacophore models and to develop nicotinic agents with reduced side effects (review, for example, refs 3 and 5−7).

Chart 1



Some of the first studies undertaken were directed toward understanding the binding requirements of nicotine itself. Modification or abbreviation of the nicotine structure has resulted in agents that retain some or all of nicotine's actions or that retain affinity for nACh receptors.[7] Nevertheless, most simple modifications of the nicotine structure result in decreased affinity. For example, with respect to binding, the intact structure of nicotine (**1**; K_i ca. 2−5 nM; Chart 1) is not required but appears optimal.[6,7] Ring opening of **1** results in a series of 3-(aminomethyl)pyridines (AMPs; **2**) with varying affinities. The simple *N*,*N*-dimethyl AMP **2a** (K_i = 540 nM) binds with much lower affinity than nicotine. However, further structural manipulation of the AMPs can result in enhanced affinity; homologation of one of the *N*-methyl groups of **2a** to the *N*-ethyl-*N*-methyl derivative **2b** (K_i = 28 nM), for example, results in substantially improved affinity. Extension of the side chain of **2a** to the 3-(2-aminoethyl)-pyridine **3** (K_i = 47 nM) also increases affinity by an

* To whom correspondence should be addressed. Tel: 804-828-8487. Fax: 804-828-7404. E-mail: glennon@hsc.vcu.edu.
† Universita di Camerino.
‡ Department of Medicinal Chemistry, Virginia Commonwealth University.
§ Department of Pharmacology and Toxicology, Virginia Commonwealth University.

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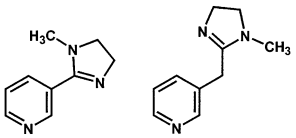
order of magnitude whereas further extension to **4** (K_i > 10 000 nM) results in loss of affinity.[7]

Introduction of unsaturation, as with **5** (K_i = 2775 nM), reinstates some of the affinity lost with **4**; however, replacement of the benzylic sp^2-hybridized carbon atom of **5** with an ether oxygen atom once again reestablishes high-affinity binding. For example, **6** (K_i = 21 nM) binds with 25 times the affinity of **2a** (review refs 6 and 7). Abbott Laboratories has independently developed a number of related pyridyl ethers as high-affinity nACh receptor agonists[8] (review refs 3 and 7).

In the course of our studies, we examined several aza analogues of nicotine such as azanicotine (**7**) and homoazanicotine (**8**).[9] Compound **7** might be viewed as an analogue of **2** much in the same manner that **8** is an analogue of **3**. Although azanicotine (**7**; K_i = 206 nM) binds with modest affinity, homoazanicotine (**8**; K_i = 7.8 nM) binds with an affinity comparable to that of nicotine.[9] As a result of this finding, we undertook two investigations. First, we wished to determine if **8** binds at muscarinic ACh receptors and because **8** possesses an imidazoline moiety common to certain adrenergic and serotonergic agents, whether it binds at adrenergic or serotonergic receptors. If **8** were to bind with high affinity at one of these receptors populations, an investigation of additional analogues might not be a worthwhile endeavor. Second, and contingent upon the results of the first study, we proposed to undertake an investigation of the SAFIR of **8**. In particular, because preliminary indications suggested that **7** and **8** might bind differently than nicotine at nACh receptors,[9] we wished to determine if **8** behaves in a manner similar to that of nicotine and nicotine analogues **4**−**6**. Other structure−affinity features were also examined.

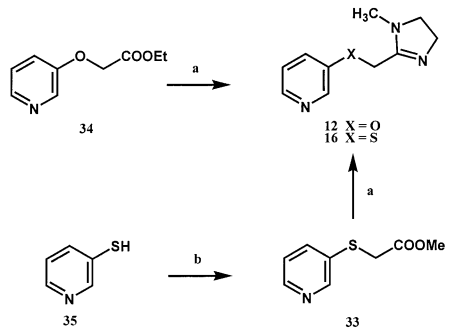


7 8

Chemistry

The synthesis of **7** and **8** was previously reported.[9] Most of the compounds in the present study were prepared in a similar manner. That is, a requisite pyridine bearing a 3-position substituent possessing an ester function (e.g., a pyridine-3-carboxylate, a pyridine-3-acetate) was allowed to react with Me_3Al and an appropriately substituted diamine (e.g., ethylenediamine). For example, reaction of **34** with *N*-methylethylenediamine in the presence of Me_3Al provided **12** (Scheme 1). Compounds **9**, **10**, **13**, **16**, **18**−**22**, and **24**−**26** (Table 1) were prepared in this manner. Compounds **28a**,**b** were prepared in the same manner using the methyl esters of cinnamic acid and 3-chlorocinnamic acid, respectively. In most instances, the requisite pyridyl ester component was readily available; in some cases, such as for **16** (Scheme 1) and **20**,[10] it was necessary to prepare the ester. Compound **28c** was prepared by cyclization of methyl 3-nitrocinnamate followed by catalytic reduction of the product to the amine. Compound **31** was prepared from the appropriately 4-substituted pyridine.

Scheme 1[a]



[a] Reagents and conditions: (a) Me_3Al, toluene, CH_3-NH-CH_2CH_2-NH_2. (b) Cl−CH_2COOMe.

Table 1. Physicochemical Properties of Homoazanicotine Analogues[a]

	mp (°C)	recryst solvent	% yield	empirical formula[b]
9	153−155	95% EtOH	20	$C_{11}H_{15}N_3 \cdot 2C_2H_2O_4 \cdot H_2O$
10	95−97	abs EtOH	22	$C_{12}H_{17}N_3 \cdot 2C_2H_2O_4$
12	167−168	95% EtO H	47	$C_{10}H_{13}N_3O \cdot 2C_2H_2O_4$
13	180−181	abs EtOH	28	$C_9H_{12}N_4 \cdot C_2H_2O_4$
16[c]	109−111	2-PrOH	27	$C_{10}H_{13}N_3S \cdot 2C_2H_2O_4 \cdot 1.25H_2O$
18	198−200	MeOH/EtOAc	40	$C_8H_9N_3 \cdot C_2H_2O_4$
19	180−182	abs EtOH	65	$C_9H_{11}N_3 \cdot 2C_2H_2O_4$
20[d]	135−136	abs EtOH	36	$C_{11}H_{15}N_3 \cdot 2C_2H_2O_4$
21	168−169	MeOH/EtOAc	68	$C_{11}H_{15}N_3 \cdot 2C_2H_2O_4$
22	113−114	MeOH/EtOAc	83	$C_{16}H_{17}N_3 \cdot 2C_2H_2O_4$
24	230−232	95% EtOH	10	$C_{12}H_{15}N_3 \cdot 2HCl \cdot 0.5H_2O$
25	162−163	2-PrOH	9	$C_{17}H_{17}N_3 \cdot 2C_2H_2O_4$
26	200−202	abs EtOH	48	$C_9H_{11}N_3O \cdot 2C_2H_2O_4$
28a	151−152	2-PrOH/Et_2O	30	$C_{12}H_{14}N_2 \cdot 2C_2H_2O_4 \cdot 1.25H_2O$
28b	177−178	abs EtOH	15	$C_{12}H_{13}ClN_2 \cdot C_2H_2O_4$
31	176−178	abs EtOH	30	$C_{11}H_{13}N_3 \cdot 2C_2H_2O_4$

[a] Compounds were prepared in a manner described in detail for **12** (see Experimental Section). [b] Compounds analyzed for C, H, and N within 0.4% of theory. [c] Compound **33** was used as starting material. [d] Methyl 2-(3-pyridyl)propanoate[10] was employed as starting material.

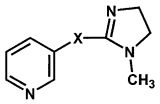
Compounds **11** and **23** were prepared by condensation of pyridine-3-carboxaldehyde with *N*-methyl-2-methylimidazoline and 2-methylimidazaoline, respectively. Epoxides **17***E*,*Z* were prepared from the individual isomers of 2-(3-pyridyl)-2,3-epoxypropionitrile[11] by condensation with *N*-methylethylenediamine. Amidines **27** were obtained by reaction of pyridyl-3-acetonitrile with sodium methoxide and the appropriately substituted amine hydrochloride; compound **27c** had been previously reported.[12]

Results and Discussion

Binding Profile of Compound 8. Compound **8** represents the first member of a novel class of nicotinic cholinergic ligands.[9] Because **8** possesses an imidazoline ring and because imidazoline derivatives can bind at certain other types of neurotransmitter receptors, we examined the selectivity of **8** at several relevant receptors. Compound **8** displayed no affinity (K_i > 10 000 nM) for any of the receptors examined including m_1−m_5 muscarinic cholinergic receptors, 5-HT_{1A}, r5-HT_{1B}, h5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_{5A}, 5-HT_6, 5-HT_7 serotonin receptors, and α_{1A}, α_{1B}, α_{2A}, α_{2B}, α_{2C}, β_1, and β_2 adrenergic receptors.[13] As such, **8** appears fairly selective for nACh receptors.

Having established the selective nature of **8**, we next examined the SAFIR for the binding of **8** and related

Table 2. Effect of Altering the Pyridine-to-Amine Connector on the Binding of **8**



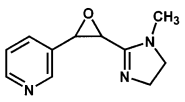
	X	nACh receptor affinity; K_i (±SEM) (nM)
8	$-CH_2-$	7.8[a]
9	$-CH_2-CH_2-$	95 (7)
10	$-CH_2-CH_2-CH_2-$	4170 (1110)
11	$-CH=CH-$	62 (2)
12	$-O-CH_2-$	57 (12)
13	$-NH-$	94 (5)
14	$-NH-CH_2-$	310 (40)
15	$-CH_2-NH-$	420 (55)
16	$-S-CH_2-$	4100 (1300)

[a] K_i value previously reported.[9]

compounds at nACh receptors. In particular, we focused on those structural modifications known to have an effect on the binding of nicotine and compounds such as **2**−**6** including the influence of the length and composition of the pyridyl ring-to-amine connector, terminal amine substituents, ring opening, and the necessity of the pyridyl nitrogen atom. Aromatization of the imidazoline ring of **8** was also explored.

Influence of the Connector. Compound **8** (K_i = 7.8 nM) binds with high affinity.[9] It has been shown that shortening the connector in the ring-opened series (i.e., **3** → **2a**) results in a 20-fold decreased affinity, whereas lengthening the connector of **3** (i.e., **4**) abolishes affinity.[14] Shortening the connector of **8** (i.e., **7**; K_i = 206 nM) reduced affinity by about 25-fold, whereas, interestingly, lengthening the connector of **8** (i.e., **9**; K_i = 95 nM; Table 2) only reduced affinity by 12-fold. Further lengthening of the connector (i.e., **10**; K_i = 4170 nM) resulted in a low-affinity compound, whereas introduction of unsaturation, as with **11** (K_i = 62 nM), had little effect on nACh receptor affinity relative to **9**.

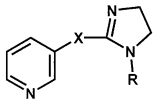
The ether analogue of **9**, compound **12** (K_i = 57 nM; Table 2), did not display the enhanced affinity seen upon conversion of **4** to **6** or upon insertion of this same type of spacer into nicotine.[7,8] Likewise, the amines **13**−**15** (K_i = 94, 310, and 420 nM, respectively) were found to bind with modest affinity and the thioether analogue, **16** (K_i = 4100 nM), with low affinity. The epoxide **17** was also examined; **17***Z* (K_i > 10 000 nM) lacked affinity whereas **17***E* displayed low affinity (K_i = 540 ± 60 nM) relative to **9**.



17 (*E* and *Z*)

Terminal Amine Substituents. Demethylation of nicotine analogues typically reduces affinity, but the extent of the reduction depends on the particular nicotine analogue being examined.[7] For example, demethylation of nicotine (to nornicotine) reduces affinity by about 20−30-fold; demethylation of **2a** reduces affinity by at least the same amount.[5,15] However, demethylation of **3** reduces affinity by 6-fold, and demethylation of **5** and **6** reduces affinity only by 2-fold.[14,15] An *N*-methyl substituent appears optimal for the binding of nicotine and increasing the size of the substituents to larger alkyl substituents decreases affinity.[16] This is not true, however, for **2a** (i.e., its *N*-ethyl homologue **2b** binds with 20-fold enhanced affinity), or for **6** (i.e., its *N*-ethyl homologue binds with an affinity identical to that of **6**).[14] Evidently, the nature of the connector has an influence on the role of the *N*-substituents on binding. Hence, we examined the effect of *N*-substituents on nACh receptor affinity.

Table 3. Influence of N-Substituents on nACh Receptor Affinity



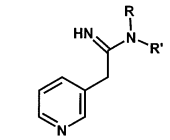
	X	R	nACh receptor affinity; K_i (±SEM) (nM)
18		$-H$	3570 (1250)
7		$-CH_3$	206[a]
19	$-CH_2-$	$-H$	325 (30)
8	$-CH_2-$	$-CH_3$	7.8[a]
20	$-CH(CH_3)-$	$-CH_3$	114 (16)
21	$-CH_2-$	$-CH_2CH_3$	150 (12)
22	$-CH_2-$	$-CH_2-Ph$	850 (90)
23	$-CH=CH-$	$-H$	>10 000
11	$-CH=CH-$	$-CH_3$	62 (2)
24	$-CH=CH-$	$-CH_2CH_3$	5010 (1000)
25	$-CH=CH-$	$-CH_2-Ph$	>10 000
26	$-O-CH_2-$	$-H$	1400 (260)
12	$-O-CH_2-$	$-CH_3$	57 (12)

[a] K_i value previously reported.[9]

N-Demethylation of azanicotine (**7**) (i.e., **18**; K_i = 3570 nM Table 3) decreased affinity by nearly 20-fold, and *N*-demethylation of homoazanicotine (**8**) (i.e., **19**; K_i = 325 nM) reduced affinity by about 40-fold. In this respect, the findings parallel those observed upon demethylation of nicotine.[16] Demethylation of the unsaturated chain analogue **11** (K_i = 62 nM) essentially abolished affinity (i.e., **23**; K_i > 10 000 nM), whereas demethylation of ether analogue **12** (i.e., **26**; K_i = 1400 nM) reduced affinity by about 25-fold. Compounds **7**, **8**, and **12**, then, behaved like nicotine rather than like ether analogue **6**; however, compound **11** with its >160-fold reduction in affinity upon demethylation behaved like none of the other nicotine analogues examined. Although the extent of affinity reduction varied, in each case, affinity was reduced upon *N*-demethylation. Introduction of an α-methyl group (i.e., **20**; K_i = 114 nM) decreased the affinity of **8** by nearly 15-fold.

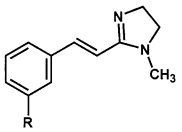
Homologation of the *N*-methyl group of nicotine to an *N*-ethyl group decreases affinity by 17-fold.[16] Homologation of the *N*-methyl group of **8** to an *N*-ethyl group (i.e., **21**; K_i = 150 nM) reduced affinity by 19-fold. The corresponding *N*-benzyl analogue of nornicotine binds with even (360-fold) lower affinity than nicotine,[16] whereas *N*-benzyl analogue **22** (K_i = 850 nM; Table 3) binds with 100-fold reduced affinity. With the unsaturated derivative **11**, the *N*-ethyl homologue binds with 80-fold reduced affinity, and the *N*-benzyl derivative binds with >100-fold reduced affinity (Table 3). Again, with respect to the ethyl homologues, the unsaturated analogue **11** seems to behave somewhat differently than nicotine or **8**. Overall, however, it can be concluded that the *N*-methyl substituents of **8** and **11** are optimal for binding.

Ring Opening. Ring opening of nicotine affords the AMPs **2**. Compound **2a**, for example, binds with substantially lower affinity than nicotine itself. Homoazanicotine (**8**) is a cyclic amidine; several homoazanicotine-related amidines were examined. Compound **27c** (K_i = 280 ± 55 nM) binds with reduced affinity. *N*-Demethylation and *N*,*N*-di-demethylation of **27c** to afford **27a**,**b** resulted in further reduction in affinity (K_i = 1510 ± 170 nM and 5820 ± 2200 nM, respectively). As with nicotine, the intact ring of **8** appears optimal for binding. However, reminiscent of the homologation of **2a** to **2b**, homologation of one of the methyl groups of **27c** to **27d** enhanced affinity by nearly 60-fold (**27d**, K_i = 4.9 ± 2.5 nM). In fact, the affinity of **27d** is very similar to that of **8**.



27a R = R' = -H
27b R = H, R' = -CH_3
27c R = R' = -CH_3
27d R = -CH_3, R' = -C_2H_5

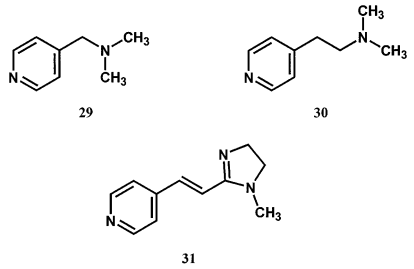
Pyridine Nitrogen Atom. With few exceptions,[5,7] replacement of the pyridyl ring of nicotine with another heterocyclic or aromatic ring considerably reduces affinity. This effect was examined with compound **11**. Replacement of the pyridyl ring of **11** with a phenyl ring (**28a**, K_i = 5450 ± 2100 nM) resulted in a substantial reduction in affinity. The 3-chlorophenyl analogue **28b** (K_i = 3460 ± 1350 nM) also binds with low affinity. Interestingly, the 3-aminophenyl analogue **28c** (K_i = 145 ± 5 nM) binds only with about 2-fold reduced affinity relative to **11** (K_i = 62 nM). It has been proposed that the pyridine nitrogen atom of nicotine acts as a hydrogen bond acceptor upon interaction with nACh receptors;[17,18] hence, its presence and position in the ring seem to play a critical role in binding. For example, replacement of the pyridine nitrogen atom of nicotine with an sp^2-hybridized carbon atom reduces affinity by about 500-fold.[15,19,20] The enhanced affinity of **28c** over **28a** might be explained by the presence of the amino group, which, although perhaps not optimally situated, could also form a hydrogen bond with the receptor. Elliot et al.[18] have used a similar argument to explain the higher affinity of a 3-fluorophenyl analogue of nicotine over N_1-deazanicotine.



28a R = -H
28b R = -Cl
28c R = -NH_2

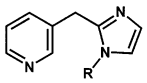
To test the importance of the position of the pyridine ring nitrogen atom, we prepared the positional isomers of **2a**, **3**, and **11** (i.e., **29**−**31**, respectively; Chart 2) for comparison. Compounds **29** and **30** (K_i > 10 000 nM) lacked affinity for nACh receptors whereas **31** (K_i = 2100 ± 880 nM) binds with reduced affinity. Although compound **30** binds with >200-fold lower affinity than **3** (K_i = 47 nM), compound **31** binds only with about 30-fold lower affinity than **11**. Here too, the direction of the effect is the same, but the magnitude differs.

Chart 2



29 **30** **31**

Aromatization of the Imidazoline Ring. The imidazoline ring of **8** and its *N*-desmethyl derivative **19** was replaced with an imidazole ring. Compound **32a** (K_i = 28 ± 3 nM), the aromatic analogue of **8**, was found to bind with slightly reduced affinity relative to **8** (K_i = 7.8 nM), and compound **32b** (K_i = 370 ± 40 nM) was found to bind with an affinity comparable to its imidazoline parent **19** (K_i = 325 nM). Apparently, aromatization has only a small effect on nACh receptor affinity for these two compounds.

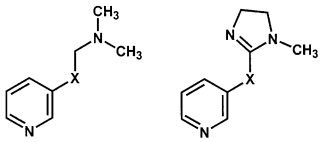


32a R = -Me
32b R = -H

Functional Studies. Compounds **7** and **8** were previously shown to produce an antinociceptive effect in the tail-flick assay in mice (ED_{50} values following intrathecal administration were 21 and 19 μg/mouse, respectively).[9] Several representative compounds, ether analogue **12**, the *N*-ethyl homologue of **8** (i.e., **21**), and the aromatic analogue of **8** (i.e., **32a**), were tested in a like manner. At the highest dose evaluated (20 μg/mouse), the compounds produced 0, 5 ± 2, and 6 ± 3%, respectively, of the maximal possible effect (MPE). Evidently, these compounds do not produce an antinociceptive effect at doses where **7** and **8** were active. All three compounds additionally produced a short-lived convulsant effect at this dose. Compounds **12** and **32a** were also evaluated as potential antagonists of (−)-nicotine-induced antinociception. Administration of **12** and **32a** (at 10 μg/mouse) in combination with (−)-nicotine (20 μg/mouse) had no effect (77 ± 15 and 74 ± 12% MPE, respectively) on the antinociceptive effect of (−)-nicotine (73 ± 15% MPE when administered alone). Although the compounds failed either to produce an antinociceptive effect or to block the antinociceptive effect of (−)-nicotine, examination of higher doses was precluded by their convulsant actions. Nicotine-induced seizures may involve α4β2 and non-α4β2 receptor subtypes. Indeed, pharmacological and genetic approaches suggested initially the involvement of α7 nicotinic subtypes in nicotine-induced seizures; however, additional studies on different mouse inbred strains have also implicated α4, α5, and α6 subunits as determinants of the sensitivity to nicotine-induced seizures.[21,22]

Summary. Compound **8** (K_i = 7.8 nM) represents a novel nACh receptor ligand. The present investigation shows that even though **8** possesses an imidazoline

Table 4. Summary of the Effect on nACh Receptor Affinity of Parallel Structural Changes in Imidazoline versus Non-imidazoline Derivatives



	K_i (nM)	X		K_i (nM)
2a	540		**7**	206
3	47	$-CH_2-$	**8**	7.8
4	>10 000	$-CH_2-CH_2-$	**9**	95
5	2775	$-CH=CH-$	**11**	62
6	21	$-O-CH_2-$	**12**	57

moiety it does not display affinity for muscarinic, serotonergic, or adrenergic receptors. Its SAFIRs for nACh receptor binding bear certain similarities to that formulated for nicotine. As with nicotine, an *N*-methyl group appears optimal for affinity; *N*-demethylation (i.e., **19**) and homologation to an *N*-ethyl group (i.e., **21**) decrease affinity, ring opening of the five-membered ring (e.g., **27**) decreases affinity (although with the appropriate amine substituents affinity can be enhanced), and the presence and location of the pyridyl nitrogen atom are an important contributors to binding. However, in comparing the affinity of ring-opened nicotine-related analogues with those of homoazanicotine-related analogues, differences seem to exist as the spacer moiety is varied. For example, comparing **4** with **6** and **9** with **12**, introduction of an ether oxygen atom does not substantially increase affinity. Furthermore, unlike what was seen with **5**, the unsaturated analogue **11** binds with higher affinity than expected, whereas unlike the ether **6**, *N*-demethylation of **12** to **26** results in an agent with reduced affinity. In fact, alteration of the connector has nonparallel effects when ring-opened nicotine analogues are compared with their corresponding imidazoline counterparts (Table 4). At this time, it cannot be concluded that the "aza" or imidazoline analogues of nicotinic agents bind at nACh receptors in a manner similar to that of the nicotine analogues themselves. Further studies will be required to address this issue. Nevertheless, as with nicotine, structural changes to homoazanicotine typically resulted in decreased affinity. Finally, during the course of these investigations, a novel class of nicotinic ligands was identified: 2-(3-pyridyl)acetamidines. Pyridylacetamidine **27d**, a ring-opened analogue of homoazanicotine (**8**), was found to bind with nearly twice the affinity of **8** and with an affinity (K_i = 4.9 nM) similar to that of nicotine. Additional studies with pyridylacetamidines are currently in progress.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Buchi SMP-20 or Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian EM-390 spectrometer, and peak positions are given in parts per million (δ) downfield from tetramethylsilane as the internal standard. Microanalyses were performed in the Microanalytical Laboratory at the University of Camerino or by Atlantic Microlab (GA) for the indicated elements, and the results are within 0.4% of the calculated values. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040−0.063 mm, Merck) by flash chromatography. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. Dry tetrahydrofuran (THF) was obtained by distillation over sodium metal and benzophenone.

3-[(*E*)-2-(1-Methyl-4,5-dihydro-1*H*-2-imidazolyl)-1-ethenyl]pyridine Trioxalate (11). A mixture of pyridine-3-carboxaldehyde (2.58 g, 24.12 mmol), *N*-methyl-2-methylimidazoline[23] (2.84 g, 28.93 mmol), and *n*-butyl formate (5.5 mL) was heated at 100 °C for 2.5 h. After the reaction mixture was cooled, the volatile components were evaporated under reduced pressure to give a residue that was purified on a silica gel column by elution with petroleum ether/EtOAc/MeOH/NH_4OH 28% (3:4:1.5:0.1). This product was converted to its oxalate salt and recrystallized from 95% EtOH to yield 0.88 g (8%) of **11**; mp 164−165 °C. ^{1}H NMR [DMSO-d_6]: δ 3.25 (s, 3H, N−CH_3), 3.70−4.10 (m, 4H, NCH_2CH_2N), 7.30 (d, 1H, J = 16.1 Hz, CH−C=N), 7.88 (d, 1H, J = 16.2 Hz, CHAr), 7.50−8.95 (m, 4H, ArH). Anal. ($C_{11}H_{13}N_3 \cdot 3C_2H_2O_4$) C, H, N.

Compound **23** was similarly obtained, in 20% yield (mp 210−211 °C after recrystallization from 95% EtOH), from commercially available pyridine-3-carboxaldehyde and 2-methylimidazoline. Anal. ($C_{10}H_{11}N_3 \cdot 2C_2H_2O_4$) C, H, N.

3-[(1-Methyl-4,5-dihydro-1*H*-imidazol-2-yl)methoxy]-pyridine Dioxalate (12). A solution of *N*-methylethylenediamine (0.98 mL, 11.03 mmol) in dry toluene (6.5 mL) was added in dropwise fashion to a vigorously stirred solution of 2 M trimethylaluminum (11.04 mL, 22.07 mmol) in dry toluene (18 mL) at 0 °C under a nitrogen atmosphere. After it was allowed to stir at room temperature for 1 h, the solution was cooled to 0 °C and a solution of ethyl-3-pyridoxyacetate[24] (1 g, 5.52 mmol) in dry toluene (9 mL) was added in a dropwise manner. The reaction mixture was heated at reflux for 1.5 h, cooled to 0 °C, and cautiously quenched by the dropwise addition of MeOH (4 mL) followed by water (0.8 mL). After $CHCl_3$ (32 mL) was added, the mixture was heated at reflux for 1 h at 70 °C to ensure precipitation of the aluminum salts. Solid Na_2SO_4 was added, and the mixture was filtered. The organic portion was evaporated under reduced pressure to give the free base as a pale yellow oil, which was purified on a silica gel column using as eluent $CHCl_3$/MeOH/NH_4OH 28% (95:5:1). The product was converted to an oxalate salt and recrystallized from 95% EtOH to yield 1.92 g (47%) of the product; mp 167−168 °C. ^{1}H NMR [DMSO-d_6]: δ 3.10 (s, 3H, N−CH_3), 3.80−4.10 (m, 4H, NCH_2CH_2N), 5.30 (s, 2H, OCH_2), 7.40−8.50 (m, 4H, ArH). Anal. ($C_{10}H_{13}N_3O \cdot 2C_2H_2O_4$) C, H, N.

Similarly, compounds in Table 1 were obtained from the appropriate esters prepared as previously described.[25−27] Methyl nicotinate, ethyl 3-pyridyl acetate, *trans*-cinnamic acid, methyl 3-(3-pyridyl)propionate, *N*-ethylethylenediamine, ethylenediamine, and *N*-benzyl ethylenediamine were commercially available. See Table 1 for physicochemical data on compounds **9**, **10**, **13**, **16**, **18**−**22**, **24**−**26**, **28a**,**b**, and **31**.

***N*-[(1-Methyl-4,5-dihydro-1*H*-imidazol-2-yl)methyl]-3-aminopyridine Dioxalate (14).** 2-(3-Pyridyl)aminoacetonitrile[28] (3.00 g, 22.5 mmol) was added to a solution of Na (0.52 g, 22.5 mmol) in MeOH (14.0 mL), and the mixture was allowed to stir at room temperature for 18 h. The reaction mixture was cooled to 0 °C, and a solution of *N*-methylethylenediamine (1.67 g, 22.5 mmol) in dry MeOH (5.6 mL) was added in a dropwise manner. After a few minutes, a 3 N solution of HCl in MeOH (7.8 mL) was added in a dropwise fashion and the mixture was allowed to warm to room temperature. After 24 h, the solid material was removed by filtration, NH_4OH (28%, 5.0 mL) was added to the filtrate, and the filtrate was evaporated to dryness. The oily residue was purified on a silica gel column by elution with $CHCl_3$/MeOH/NH_4OH 28% (90:15:1). The free base (0.65 g, 15% yield) was converted to its oxalate salt and recrystallized from 95% EtOH/MeOH; mp 152−154 °C. ^{1}H NMR [DMSO-d_6]: δ 3.08 (s, 3H, CH_3), 3.82 (m, 4H, NCH_2CH_2N), 4.39 (s, 2H, CH_2N), 6.77 (broad s, 1H, NH), 6.95−8.17 (m, 4H, ArH). Anal. ($C_{10}H_{14}N_4 \cdot 2H_2C_2O_4 \cdot 0.25H_2O$) C, H, N.

1-Methyl-*N*-(3-pyridylmethyl)-4,5-dihydro-1*H*-imidazol-2-amine (15). A mixture of 1-methyl-2-nitramino-2-imidazo-

line[29] (2.0 g, 15 mmol) and 3-aminomethypyridine (3.0 g, 28 mmol) was heated at 80 °C for 8 h. The residue was purified on a silica gel column by elution initially with $CHCl_3$/MeOH (9:1) and then with $CHCl_3$/MeOH/NH_4OH 28% (7:3:0.1) to afford 0.32 g (11%) of a solid material; mp 133−134 °C. ^1H NMR [$CDCl_3$]: δ 3.10 (s, 3H, CH_3), 3.60 (m, 4H, NCH_2CH_2N), 4.72 (d, 2H, CH_2Ar), 7.13−9.08 (m, 4H, ArH), 9.58 (t, 1H, NH). Anal. ($C_{10}H_{14}N_4 \cdot 2.5H_2O$) C, H, N.

(*Z*)-3-[3-(1-Methyl-4,5-dihydro-1*H*-imidazol-2-yl)-2-oxiranyl]pyridine Trioxalate (17*Z*). A mixture of (*Z*)-3-(3-pyridyl)-2,3-epoxypropionitrile[11] (0.755 g, 5.16 mmol), sodium methoxide (0.0024 g, 0.45 mmol), and MeOH (4 mL) was allowed to stir for 4 h. After it was cooled to 0−10 °C, a solution of *N*-methylethylenediamine (0.43 g, 5.80 mmol) in MeOH (1.7 mL) was added in a dropwise manner under vigorous stirring. After a few minutes of stirring, a solution of HCl and MeOH (0.7 mL of a solution 7.86 N, 5.50 mmol) was added and the mixture was allowed to stir at room temperature for 48 h. The solvent was removed under reduced pressure to give a residue that was purified by column chromatography, eluting with $CHCl_3$/MeOH/NH_4OH 28% (9:1:0.1); the product was converted into its oxalate salt (yield, 19%; mp 121−122 °C). ^1H NMR [DMSO-d_6]: δ 3.05 (s, 3H, N−CH_3), 3.45−3.85 (m, 4H, NCH_2-CH_2N), 4.69 (d, 1H, J = 4.46 Hz, CH−C=N), 4.81 (d, 1H, J = 4.40 Hz, ArCH), 7.40−8.70 (m, 4H, ArH). Anal. ($C_{11}H_{13}N_3O \cdot 3C_2H_2O_4$) C, H, N.

(*E*)-3-[3-(1-Methyl-4,5-dihydro-*1H*-2-imidazolyl)-2-oxiranyl]pyridine Dioxalate (17*E*). Prepared in 15% yield in a manner similar to that of **17***Z* from the appropriate nitrile,[11] the product was characterized as an oxalate salt; mp 126−127 °C. ^1H NMR [DMSO-d_6]: δ 3.10 (s, 3H, N−CH_3), 3.75−4.05 (m, 4H, NCH_2CH_2N), 4.36 (d, 1H, J = 1.62 Hz, CH−C=N), 4.59 (d, 1H, J = 1.74 Hz, ArCH), 7.95−8.90 (m, 4H, ArH). Anal. ($C_{11}H_{13}N_3O \cdot 2C_2H_2O_4$) C, H, N.

2-(3-Pyridyl)acetamidine Dioxalate (27a). A solution of pyridine-3-acetonitrile (1.00 g, 8.50 mmol), Na (0.02 g, 0.86 mmol), and dry MeOH (8 mL) was allowed to stir at room temperature for 14 h. After the reaction mixture was cooled to 0−10 °C, NH_4Cl (0.53 g, 10.00 mmol) was added with stirring, and the reaction mixture was allowed to stir at room temperature for 12 h. The suspension was filtered; the filtrate was evaporated under reduced pressure, and the residue was washed several times with dry Et_2O to remove the unreacted nitrile. The remaining solid was dissolved in absolute EtOH; the solution was filtered, and the filtrate was evaporated in vacuo to afford 0.50 g (44%) of **27a** as its free base. The free base was converted to an oxalate salt; mp 190−193 °C after recrystallization from absolute EtOH. ^1H NMR [DMSO-d_6]: δ 3.73 (s, 2H, CH_2); 7.40−8.62 (m, 4H, ArH); 9.15 (s, 2H, NH_2, D_2O exchangeable); 9.43 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR [DMSO-d_6]: 35.60, 124.21, 130.45, 136.70, 149.19, 150.24, 163.40. Anal. ($C_7H_9N_3 \cdot 2C_2H_2O_4$) C, H, N.

***N*-Methyl-2-(3-pyridyl)acetamidine Dihydrochloride (27b).** Compound **27b** was prepared in 55% yield from pyridine-3-acetonitrile and $CH_3NH_2 \cdot HCl$ in a manner similar to that of **27a**. The free base was converted to a hydrochloride salt and recrystallized from *i*-PrOH/MeOH; mp 214−216 °C. ^1H NMR [DMSO-d_6]: δ 2.81−2.82 (d, 3H, J = 4.89 Hz, N−CH_3); 4.09 (s, 2H, CH_2); 7.97−8.91 (m, 4H, ArH); 9.09 (broad s, 1H, NH, D_2O exchangeable); 9.81 (broad s, 1H, NH, D_2O exchangeable); 10.44 (broad s, 1H, NH, D_2O exchangeable). ^{13}C NMR [DMSO-d_6]: 29.18, 35.33, 124.18, 130.73, 137.00, 148.99, 150.18, 161.81. Anal. ($C_8H_{11}N_3 \cdot 2HCl$) C, H, N.

***N*,*N*-Dimethyl-2-(3-pyridyl)acetamidine Dihydrochloride (27c).** Compound **27c** was prepared in 38% yield from pyridine-3-acetonitrile and $(CH_3)_2NH_2 \cdot HCl$ in a manner similar to that of **27a**. The free base was converted to an ivory-colored hydrochloride salt; mp 215−218 °C after recrystallization from absolute EtOH/Et_2O. ^1H NMR [DMSO-d_6]: δ 3.10 (s, 3H, N−CH_3); 3.14 (s, 3H, N−CH_3); 4.34 (s, 2H, CH_2); 8.00−8.96 (m, 4H, ArH); 9.02 (broad s, 1H, NH, D_2O exchangeable); 9.81 (broad s, 1H, NH, D_2O exchangeable). ^{13}C NMR [DMSO-d_6]: 33.48, 40.59, 127.14, 132.59, 142.48, 143.44, 144.84, 163.94. Anal. ($C_9H_{13}N_3 \cdot 2HCl \cdot 0.25H_2O$) C, H, N.

***N*-Ethyl-*N*-methyl-2-(3-pyridyl)acetamidine Dihydrochloride (27d).** Compound **27d** was prepared in 7% yield from pyridine-3-acetonitrile and *N*-ethyl-*N*-methylamine·HCl in a manner similar to that of **27a**. The free base was converted to a hydrochloride salt and recrystallized from absolute EtOH/anhydrous Et_2O; mp 224−226 °C. ^1H NMR [DMSO-d_6]: δ 0.98−1.13 (m, 3H, CH_3); 3.06−3.11 (m, 3H, N−CH_3); 3.49−3.50 (m, 2H, CH_2); 7.90−7.95 (m, 1H, ArH); 8.36−8.38 (d, 1H, Ar); 8.82 (s, 1H, Ar); 9.08 (s, 1H, NH, D_2O exchangeable); 9.72 (s, 1H, NH, D_2O exchangeable). Anal. ($C_8H_{11}N_3 \cdot 2HCl$) C, H, N.

3-[(*E*)-2-(1-Methyl-4,5-dihydro-*1H*-2-imidazolyl)-1-ethenyl]aniline Dioxalate (28c). The precursor compound, 1-methyl-2-[(*E*)-2-(3-nitrophenyl)-1-ethenyl]-4.5-dihydro-*1H*-imidazole oxalate, was prepared via a standard method from methyl 3-nitrocinnamate[30] and purified on a silica gel column by elution with EtOAc/EtOH/NH_4OH 28% (8:2:0.1). This product, obtained in 10% yield, was characterized as its oxalate salt after recrystallization from MeOH/H_2O; mp 192−195 °C. ^1H NMR [$CDCl_3$]: δ 2.85 (s, 3H, N−CH_3), 3.30 (t, 2H, CH_2N), 3.70 (t, 2H, CH_2N), 6.65 (d, 1H, J = 16.0 Hz, CH−C=N), 7.52 (d, 1H, J = 16.2 Hz, ArCH), 7.40−8.25 (m, 4H, ArH). A solution of 1-methyl-2-[(*E*)-2-(3-nitrophenyl)-1-ethenyl]-4,5-dihydro-*1H*-imidazole oxalate (0.75 g, 3.24 mmol) in MeOH (15 mL) and an excess of oxalic acid (0.36 g, 4 mmol) was hydrogenated for 8 h at room temperature under pressure (20 psi) using a catalytic amount of Raney Ni. Following removal of the catalyst, evaporation of the solvent gave a mixture that was purified by recrystallization from 95% EtOH/MeOH to give a 15% yield of **28c**; mp 168−170 °C. ^1H NMR [DMSO-d_6]: δ 3.22 (s, 3H, N−CH_3), 3.80−4.00 (m, 4H, NCH_2CH_2N), 6.65−7.20 (m, 4H, ArH), 6.92 (d, 1H, J = 16 Hz, CH−C=N), 7.71 (d, 1H, J = 16 Hz, ArCH), 8.50 (broad s, 2H, NH_2, D_2O exchangeable). Anal. ($C_{12}H_{15}N_3 \cdot 2C_2H_2O_4$) C, H, N.

4-(*N*,*N*-Dimethylaminomethyl)pyridine Dioxalate (29). A mixture of pyridine-4-carboxaldehyde (1.18 g, 11 mmol), *N*,*N*-dimethylamine (0.5 g of gas in 10 mL of MeOH), and a catalytic amount of 10% Pd/C was hydrogenated at 50 psi for 1 h. The catalyst was removed by filtration, and the filtrate was evaporated to a viscous oil. A mixture of the oil and H_2O (20 mL) was extracted with Et_2O (3 × 25 mL); the combined ethereal extract was dried (K_2CO_3), and the solvent was removed in vacuo to afford 0.38 g (28%) of **29** as its free base after short-path distillation (bp 52−53 °C). The oxalate salt was obtained as fine white needles, mp 149−150 °C after recrystallization from absolute EtOH. The product was used without further characterization. Anal. ($C_8H_{12}N_2 \cdot 2C_2H_2O_4$) C, H, N.

4-(*N*,*N*-Dimethylaminoethyl)pyridine Dioxalate (30). A solution of 4-vinylpyridine (2.1 g, 20 mmol) and dimethylamine hydrochloride (3.26 g, 40 mmol) in MeOH (20 mL) was heated at reflux for 8 h. Solvent was removed under reduced pressure, and the residue was poured onto crushed ice (100 g). The mixture was made basic (pH 11) with 10% NaOH solution and extracted with Et_2O (4 × 50 mL); the combined extracts were dried (Na_2SO_4) and filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (silica gel) with CCl_3/MeOH (9:1) as eluent to give 2.65 g (75%) of the product. The oil was converted to an oxalate salt and recrystallized from 95% EtOH; mp 159 °C. ^1H NMR [free base, $CDCl_3$]: δ 2.25 (s, 6H, NMe_2), 2.60 (t, 2H, $-CH_2$-), 2.75 (t, 2H, $-CH_2N$), 7.15 (d, 2H, ArH), 8.45 (d, 2H, ArH). Anal. ($C_9H_{14}N_2 \cdot 2C_2H_2O_4 \cdot 0.75$ H2O) C, H, N.

3-[(1-Methyl-1*H*-imidazol-2-yl)methyl]pyridine Dioxalate (32a). Sodium hydride (60% suspension in mineral oil, 40 mg, 1.66 mmol) was added to a stirred, cold (ice bath) solution of 3-(1*H*-imidazol-2-ylmethyl)pyridine (154 mg, 0.97 mmol) in anhydrous THF (10 mL). After 5 min, iodomethane (0.066 mL, 1.06 mmol) was added, and the mixture was allowed to stir at room temperature for 30 min. The solvent was removed under reduced pressure, the residue was taken up in NaOH 2 N (3 mL), and the solution was extracted with EtOAc (2 × 25 mL). The combined extracts were dried (Na_2-

SO_4) and filtered, and the solvent was evaporated under reduced pressure to afford a yellow oil (145 mg), which was purified on a silica gel column by elution with $CHCl_3$/EtOAc/EtOH (8.5:1:0.5) to give the target compound as its free base (70 mg; 42%). The free base was converted to its oxalate salt and recrystallized from MeOH/EtOAc; mp 170−171 °C. ^{1}H NMR [DMSO-d_6]: δ 3.72 (d, 3H, J = 3.52 Hz, N−CH_3); 4.37 (s, 2H, CH_2); 7.36−7.42 (m, 2H, HC=CH); 7.50−8.55 (m, 4H, ArH). Anal. ($C_{10}H_{11}N_3 \cdot 2C_2H_2O_4$) C, H, N.

3-(1*H*-Imidazol-2-ylmethyl)pyridine Dioxalate (32b). Compound **27a** (free base; 0.20 g, 0.96 mmol) was suspended in anhydrous MeOH (2 mL); aminoacetaldehyde dimethylacetal (0.20 mL, 1.84 mmol) was added, and the reaction mixture was heated at reflux for 16 h and cooled, and the solvent was evaporated under reduced pressure. The crude mixture was dissolved in absolute EtOH and layered with anhydrous Et_2O to form a precipitate. The solid material was removed by filtration, and the solvent was evaporated to afford a pale yellow oil. The oil was dissolved in 1 M oxalic acid solution with heating at 80 °C for 3 h. After the solution had cooled, the mixture was extracted with EtOAc to remove impurities, made basic by the addition of solid K_2CO_3, and extracted with CH_2Cl_2. The solution was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give the free base as an amber-colored gummy material. Purification was achieved using a silica gel column by elution with EtOAc/EtOH (8:2) to afford the target compound as its free base (0.048 g, 32%). The product was converted to its oxalate salt and recrystallized from MeOH/EtOAc; mp 207−209 °C. ^{1}H NMR [DMSO-d_6]: δ 4.36 (s, 2H, CH_2); 7.40 (dd, J = 4.84, 7.91, 1H, ArH); 7.49 (s, 2H, CH=CH); 7.72−8.59 (m, 3H, ArH). Anal. ($C_9H_9N_3 \cdot 2C_2H_2O_4$) C, H, N.

Methyl 2-(3-Pyridyl)mercaptoacetate (33). Methyl chloroacetate (3.25 g, 0.03 mol) was added to a solution of the sodium salt of 3-mercaptopyridine[31] (4.0 g, 0.03 mol) (i.e., **35**) in 1,2-dimethoxyethane (50 mL). The reaction mixture was allowed to stir at room temperature for 12 h. The solvent was removed, and the residue was triturated with $CHCl_3$ (50 mL). The solid was removed by filtration, and the filtrate was evaporated to dryness. The residue was purified on a silica gel column by elution with cyclohexane/EtOAc (1:1) to afford **33** (3.5 g; 68% yield) as an oil. ^{1}H NMR [DMSO-d_6]: δ 3.35 (s, 2H, SCH_2), 3.55 (s, 3H, CH_3), 7.21−8.15 (m, 4H, ArH). The product was used without further characterization in the synthesis of **16**.

Radioligand Binding Assay. Male Sprague−Dawley rats (175−225 g) obtained from Harlan Laboratories (Indianapolis, IN) were used. The animals were housed individually in an AALAC-approved facility and had free access to food and water. The study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. The receptor binding assay was conducted as previously reported in greater detail.[32,33] In brief, rat brain without cerebellum was homogenized in 10 volumes of ice-cold 0.05 M sodium potassium phosphate buffer (pH 7.4) and centrifuged at 17 500*g* (4 °C) for 30 min. The pellet was resuspended in 20 volumes of ice-cold glass-distilled water and allowed to incubate on ice for 60 min prior to centrifugation as described above. The final pellet was resuspended (40 mg/mL) in buffer; [^{3}H](−)-nicotine was incubated with 0.5 mL of tissue homogenate (final volume 1 mL) for 2 h at 4 °C, and samples were rapidly filtered through Whatman GF/C filters. Specific binding was defined as the difference in the amount of binding in the presence and absence of 100 μM (−)-nicotine tartrate. Following buffer wash, the filters were air-dried and placed in scintillation vials, and radioactivity was quantified. Following transformation of the data by the Scatchard method, the K_D and B_{max} values were determined using the program LIGAND.[34] Displacement of [^{3}H](−)-nicotine binding at 1 nM was determined in the presence of increasing concentrations of test compound and converted to percent displacement of specific binding. IC_{50} values were determined from a plot of the log concentration vs percent displacement and converted to K_i values by the method of Cheng and Prusoff.[35] K_i values were determined at least in triplicate.

Tail-Flick Test. Male ICR mice (20−25 g), obtained from Harlan Laboratories, were used. The animals were housed in groups of six and had free access to food and water. Antinociception was assessed by the tail-flick method of D'Amour and Smith as modified by Dewey et al.[36] A control response (2−4 s) was determined for each mouse before treatment, and a test latency was determined after drug administration. To minimize tissue damage, a maximum latency of 10 s was imposed. Antinociceptive response was calculated as percent MPE, where %MPE = [(test − control)/(10 − control)] × 100. Groups of 8−12 animals were used for each dose and for each treatment. The mice were tested 5 min after intrathecal injections of the nicotine analogues. Antagonism studies were carried out by pretreating the mice with either saline or drug at different times before (−)-nicotine. The animals were tested 5 min after administration of drug.

Intrathecal injections were performed free-hand between the L5 and the L6 lumbar space in unanesthetized male mice according to the method of Hylden and Wilcox.[37] The injection was performed using a 30-gauge needle attached to a glass microsyringe. The injection volume in all cases was 5 μL. Accurate placement of the needle was evidenced by a quick flick of the mouse's tail. In protocols where two sequential injections were required in an animal, the flicking motion of the tail could be elicited with the subsequent injection.

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